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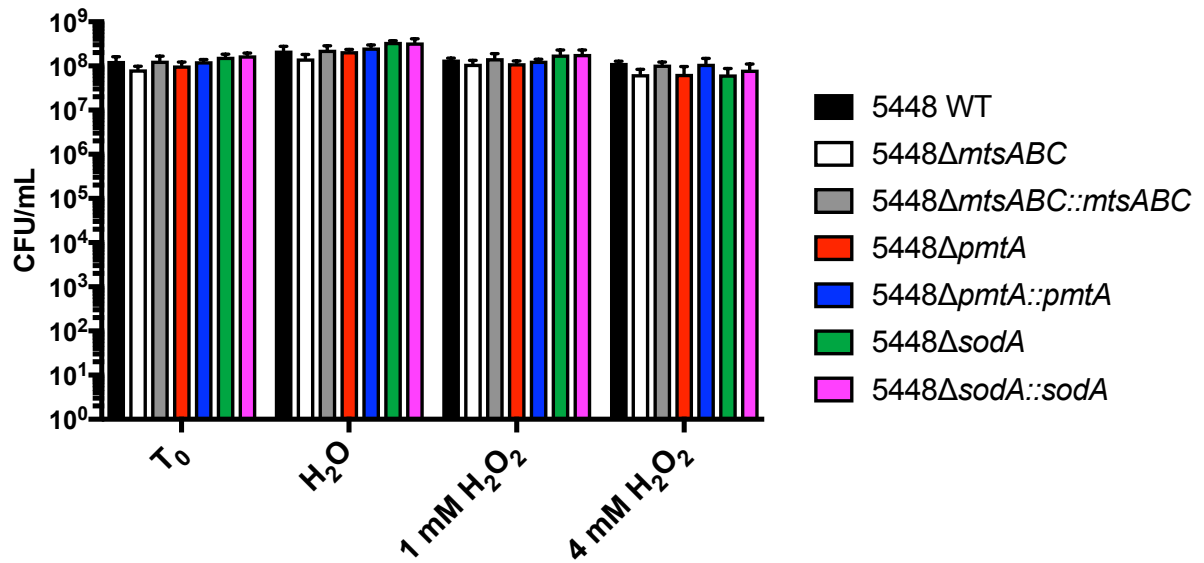
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**Table S1**

<b>Bacterial strains</b>	<b>Description</b>	<b>Reference</b>
<u><i>Escherichia coli</i></u>		
Top10	<i>E. coli</i> laboratory cloning strain	Invitrogen
Top10+pBAD- <i>sodA</i> -TEV-His	<i>E. coli</i> strain for expression of rSodA	This study
<u><i>Streptococcus pyogenes</i></u>		
5448	<i>S. pyogenes</i> invasive M1T1 strain	(1)
5448Δ <i>pmtA</i>	<i>S. pyogenes</i> 5448Δ <i>pmtA</i> :: <i>aphA</i> -3 deletion mutant	(2)
5448Δ <i>pmtA</i> :: <i>pmtA</i>	<i>S. pyogenes</i> 5448 <i>pmtA</i> complemented strain	(2)
5448Δ <i>perR</i>	<i>S. pyogenes</i> 5448Δ <i>perR</i> :: <i>aad9</i> deletion mutant	(3)
5448Δ <i>perR</i> :: <i>perR</i>	<i>S. pyogenes</i> 5448Δ <i>perR</i> complemented strain	(3)
5448Δ <i>mtsR</i>	<i>S. pyogenes</i> 5448Δ <i>mtsR</i> :: <i>aad9</i> deletion mutant	This study
5448Δ <i>mtsR</i> :: <i>mtsR</i>	<i>S. pyogenes</i> 5448Δ <i>mtsR</i> complemented strain	This study
5448Δ <i>mtsABC</i>	<i>S. pyogenes</i> 5448Δ <i>mtsABC</i> :: <i>aphA</i> -3	This study
5448Δ <i>mtsABC</i> :: <i>mtsABC</i>	<i>S. pyogenes</i> 5448Δ <i>mtsABC</i> complemented strain	This study
5448Δ <i>sodA</i>	<i>S. pyogenes</i> 5448Δ <i>sodA</i> :: <i>aphA</i> -3 deletion mutant	This study
5448Δ <i>sodA</i> :: <i>sodA</i>	<i>S. pyogenes</i> 5448Δ <i>sodA</i> complemented strain	This study
<b>Plasmids</b>		
pJRS233	Temperature sensitive shuttle plasmid::Em <sup>R</sup>	(4)
pLZ12-TS	Temperature sensitive shuttle plasmid::Spec <sup>R</sup>	This study
pUC4QKm2	Template for <i>Km</i> <sup>R</sup>	(5)
pUCSpec	Template for <i>Spec</i> <sup>R</sup>	(6)
pBAD-Myc-His-A	Template plasmid for generation of pBAD-TEV-His	This study
pBAD-TEV-His		
pBAD- <i>sodA</i> -TEV-His	pBAD-TEV-His vector with <i>sodA</i> inserted at <i>Nco</i> I and <i>Apa</i> I site	This study
pJRS233- <i>mtsR</i> -KO	pJRS233 + <i>mtsR</i> knockout construct inserted at the <i>Xho</i> I and <i>Bam</i> HI site	This study
pJRS233- <i>mtsR</i> -comp	pJRS233 + <i>mtsR</i> complement construct inserted at the <i>Xho</i> I and <i>Bam</i> HI site	This study
pJRS233- <i>mtsABC</i> -KO	pJRS233 + <i>mtsABC</i> knockout construct inserted at the <i>Xho</i> I and <i>Pst</i> I site	This study
pJRS233- <i>mtsABC</i> -comp	pJRS233 + <i>mtsABC</i> complement construct inserted at the <i>Xho</i> I and <i>Pst</i> I site	This study
pLZ12-TS- <i>sodA</i> -KO	pLZ12-TS + <i>sodA</i> KO construct	This study
pJRS233- <i>sodA</i> -comp	pJRS233 + <i>sodA</i> complement construct	This study

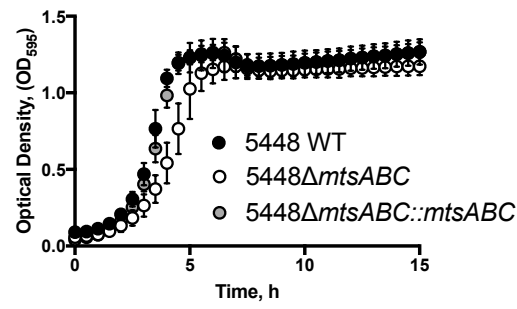
Table S2

Primer	Sequence (5'-3')	
<i>Deletion mutation and reverse complementation constructs</i>		
pBAD-site-F	GGGAATTCGAAGCTTGGGCCCCGAGGATCTGTACTTTCAGAGCGTCGACCATCATCATCATCA	
pBAD-site-R	ATATGGTACCAGCTGCAGATCTCGA	
sodA-prot-F	CCCGGG <b>CCATGGC</b> TATTATTTTACCAGAACTTCC	<i>NcoI</i>
sodA-prot-R	CGCGCG <b>GGGCC</b> TTTAGCGGCTTGGTAAAGTTCT	<i>ApaI</i>
sodA-KO-1	<u>TGGTGTTTTATGGCTCTCTTGGTCGTCAGACTGATGGGCCCTGATGCGGCTAGAG</u> ATTTGATTCATGATT	<i>pLZ-5'</i>
sodA-KO -2	<u>CAAAGTTGGCGTATAACATACGCGTATGGAAGTTCTGG</u>	<i>aphA-3 5'</i>
sodA-KO -3	<u>TACTGGATGAATTGTTTTAGACTTTACCAAGCCGCTAAATAAGAAAAGG</u>	<i>aphA-3 3'</i>
sodA-KO -4	<u>TACGAACGGCACAGATGGTCATAACCTGAAGGAAGATCTCTGCATCCATAACA</u> GCTTGCTTAGC	<i>pLZ-3'</i>
sodA-comp-F	<u>CGGGCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATTGATGCGGCTAGAG</u> ATTTGATTCATGATT	<i>pJRS-5'</i>
sodA-comp-R	<u>CTCTAGAACTAGTGGATCCCCCGGGCTGCAGGAATTCGATTCTGCATCCATAAC</u> AGCTTGCTTAGC	<i>pJRS-3'</i>
pJRS233-F	ATCGAATTCCTGCAGCCCCG	
pJRS233-R	ATCAAGCTTATCGATACCGTCGACC	
km-sodA-F	TACCAGAACTTCCATACGCGTATGTTATACGCCAACTTTGAAAACAACCTTTGAA AAAGC	<i>aphA-3 5'</i>
km-sodA-R	ATTTAGCGGCTTGGTAAAGTCTAAAACAATTCATCCAGTAAAATATAATATTTT ATTTTCTCCCAAT	<i>aphA-3 3'</i>
mtsR-KO-1	CCCGG <b>CTCGAG</b> CCATCAGACACGGCAAAGTA	<i>XhoI</i>
mtsR-KO-2	<u>GATATGATCTTTTCATTTCCATAAAACTAAAGTCTTCTTTATTAGGCGTCAT</u>	<i>aad9 5'</i>
mtsR-KO-3	<u>GGAAATATTTCATTCTAATTGGTAATCAGCTTTATGTCACAGCCCTTTAA</u>	<i>aad9 3'</i>
mtsR-KO-4	CGCGCG <b>GGATCC</b> GAATTGCAATCATTGACCAA	<i>BamHI</i>
spec-F	CTTTAGTTTTTATGGAAATGAAAGATCATATC	
spec-R	CTGATTACCAATTAGAATGAATATTTCC	
mtsABC-KO-1	CCCGGG <b>CTCGAG</b> TCGTTTGGCCTTTCTCTTATT	<i>XhoI</i>
mtsABC-KO-2	<u>CAAAGTTGGCGTATAACATACAACATCTTCTGGTAATGGTTC</u>	<i>aphA-3 5'</i>
mtsABC-KO-3	<u>TTACTGGATGAATTGTTTTAGTATTGATTACGCCAGCAGCGACA</u>	<i>aphA-3 3'</i>
mtsABC-KO-4	CCCGGG <b>CTGCAG</b> CTTATCAGATAATAGGCTAAATACC	<i>PstI</i>
km-F	TATGTTATACGCCAACTTTG	
km-R	CTAAAACAATTCATCCAGTAA	
pLZ-12-F	AGATCTTCCTTCAGGTTATG	
pLZ-12-R	GGGCCCATCAGTCTGACG	
<i>Gene expression studies</i>		
<i>pmtA</i> -qRT-F	GAAAAGCAAAACCGCCACCT	
<i>pmtA</i> -qRT-R	GGGCACATGGTGAAGCTACT	
<i>sodA</i> -qRT-F	CCTGAACCAAAACGTCCAGT	
<i>sodA</i> -qRT-R	CACCTAAACCATGCGCTTTT	
<i>mtsA</i> -qRT-F	CAAACAATTGATTGCAAAGGATCC	
<i>mtsA</i> -qRT-R	GTCTAGTTTTTCCAATTTAGCCACA	
<i>dpr</i> -qRT-F	ATGCGTGGTCCAGGTTTCTT	
<i>dpr</i> -qRT-R	AATAAGGCGCTCCGCCAATA	
<i>siaA</i> -qRT-F	CACTTCGGTTGCTGTGGTTG	
<i>siaA</i> -qRT-R	ATGGGTAAACCCACACGCTT	
<i>shp</i> -qRT-F	TGGAAGCTTTTCAGGCAGTTGA	
<i>shp</i> -qRT-R	TTCAACAAACATGCTGCCTCG	
<i>shr</i> -qRT-F	GAAAGCAACAAAGACGCGGT	
<i>shr</i> -qRT-R	ACAGTCGATGAGTATCGGCG	
<i>ahpF</i> -qRT-F	TTAATGGCCGAAGTGGAGGC	
<i>ahpF</i> -qRT-R	CCATTTGGCCCCAAGAGCTA	
<i>gyrA</i> -RT-F	GAAGTGATCCCCTGGACCTGA	
<i>gyrA</i> -RT-R	CCCGACCTGTTTGTAGTTGTT	

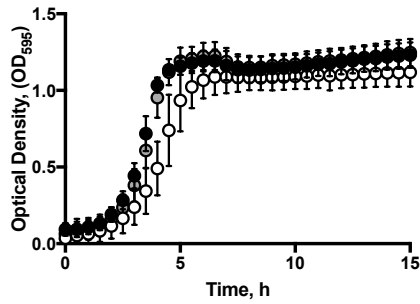


**Figure S1 - Viability of GAS strains following hydrogen peroxide challenge.** Strains 5448 WT, 5448Δ*mtsABC*, 5448Δ*mtsABC*::*mtsABC*, 5448Δ*pmtA*, 5448Δ*pmtA*::*pmtA*, 5448Δ*sodA* and 5448Δ*sodA*::*sodA* were grown in THY to mid exponential phase (OD<sub>600</sub> = 0.6) and challenged with either H<sub>2</sub>O, 1 mM H<sub>2</sub>O<sub>2</sub> or 4 mM H<sub>2</sub>O<sub>2</sub> and cell viability assessed by plating on THY agar after 30 min challenge.

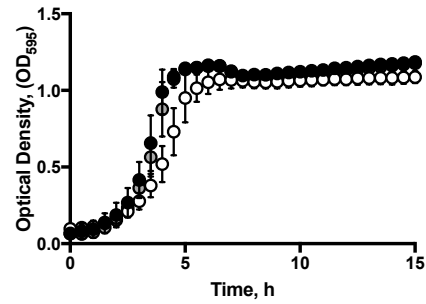
# THY



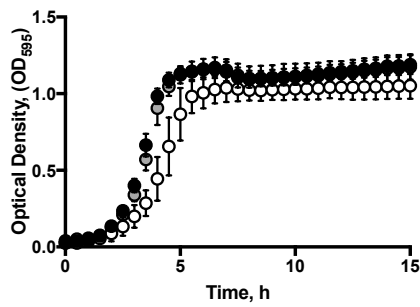
## THY + 20 μM Fe(II)



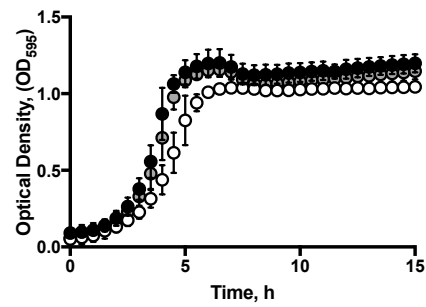
## THY + 20 μM Zn(II)



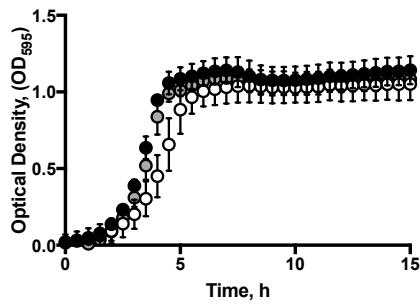
## THY + 100 μM Fe(II)



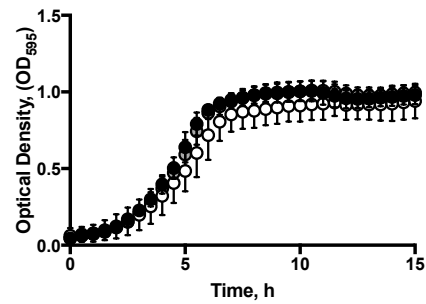
## THY + 100 μM Zn(II)



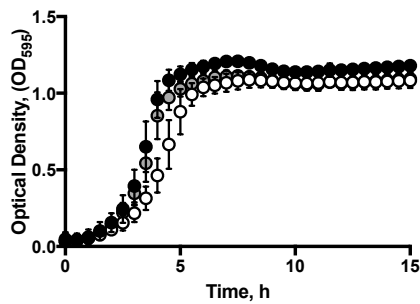
## THY + 0.5 mM Fe(II)



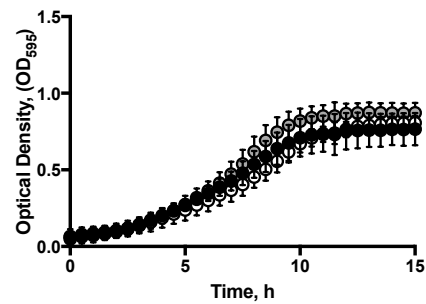
## THY + 0.5 mM Zn(II)



## THY + 1 mM Fe(II)

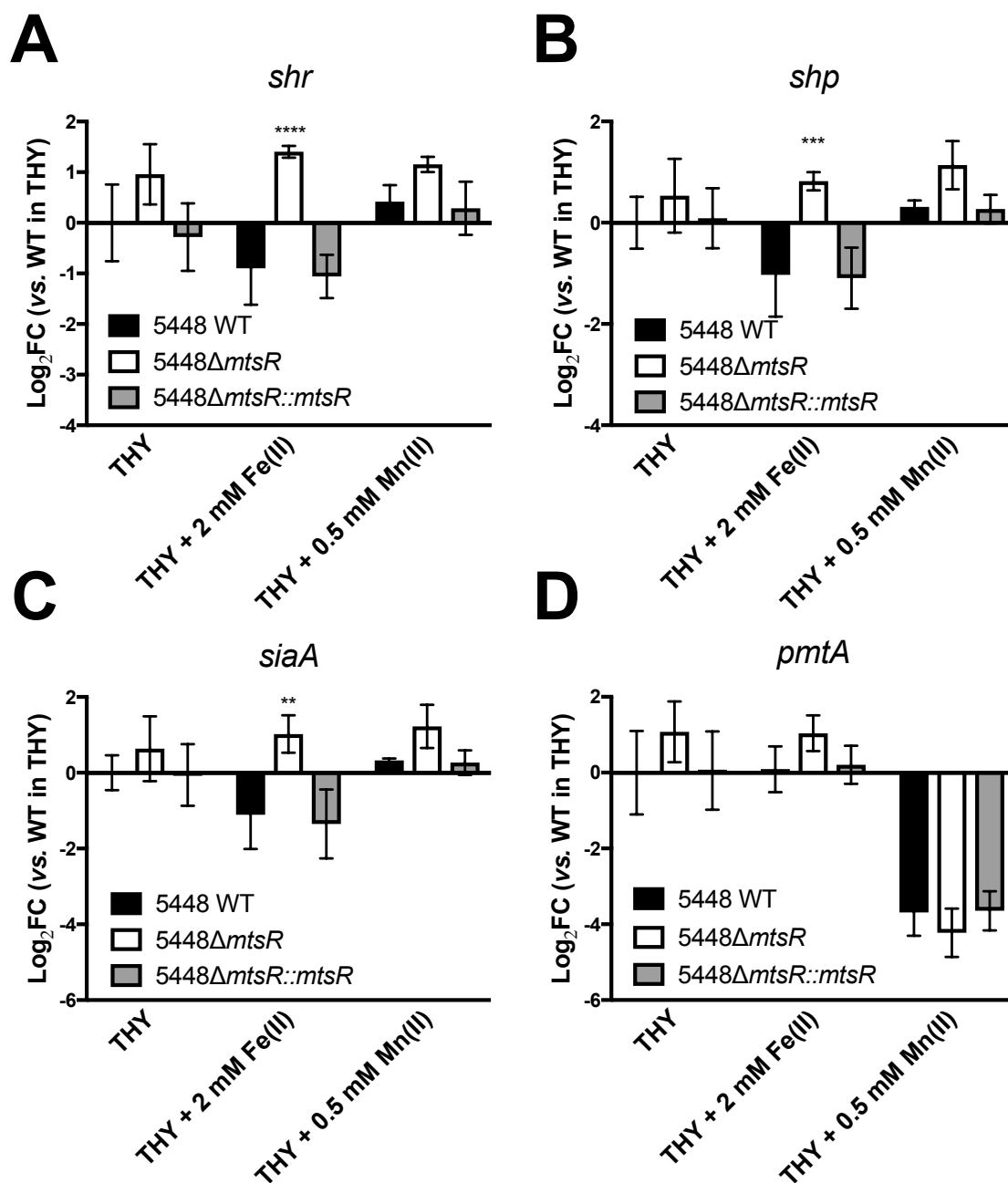


## THY + 1 mM Zn(II)



**Figure S2 - Growth curve analysis of 5448 WT, 5448 $\Delta$ *mtsABC* and 5448 $\Delta$ *mtsABC::mtsABC* in the presence of Fe(II) or Zn(II).**

Growth curve analysis of 5448 WT, 5448 $\Delta$ *mtsABC* and 5448 $\Delta$ *mtsABC::mtsABC* in the presence of Fe(II) or Zn(II). Overnight cultures of 5448 WT (black circles), 5448 $\Delta$ *mtsABC* (open circles) and 5448 $\Delta$ *mtsABC::mtsABC* (grey circles) were diluted to OD<sub>600</sub> = 0.05 into THY broth alone or THY supplemented with 20  $\mu$ M, 100  $\mu$ M, 0.5 mM or 1 mM Fe(II) or Zn(II). Growth was monitored at 37°C by optical density recording at 595 nm (OD<sub>595</sub>). Graphs represent mean  $\pm$  standard deviation of 3 independent biological replicates

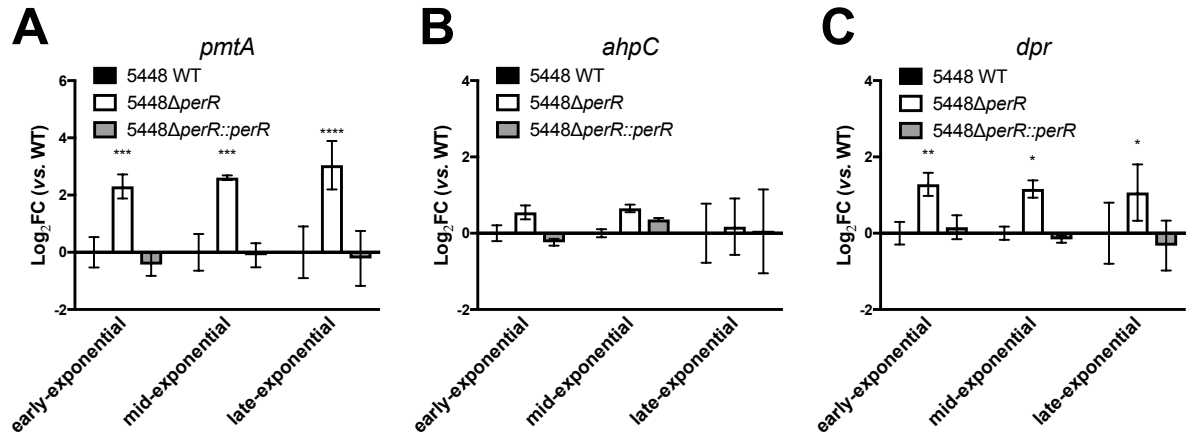


**Figure S3 - Gene expression analysis of MtsR-regulated genes in 5448 WT, 5448Δ*mtsR* and 5448Δ*mtsR*::*mtsR***

5448 WT, 5448Δ*mtsR*, and 5448Δ*mtsR*::*mtsR* were grown at 37°C to mid-exponential phase (OD<sub>600</sub> = 0.6-0.8) in either THY alone, THY + 2 mM Fe(II) or 0.5 mM Mn(II) and gene expression of *shp* (A), *shr* (B), *siaA* (C), and *pmtA* (D) analysed by qPCR using *gyrA* as the reference gene. Data represents the mean ± standard deviation of 3 independent biological

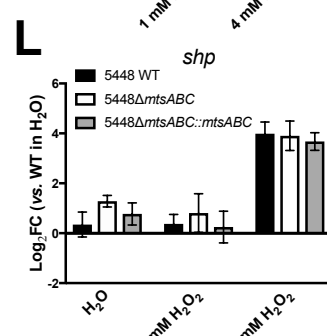
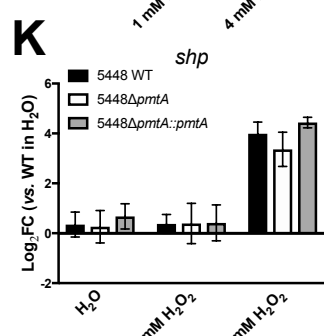
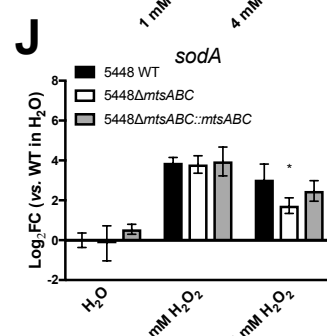
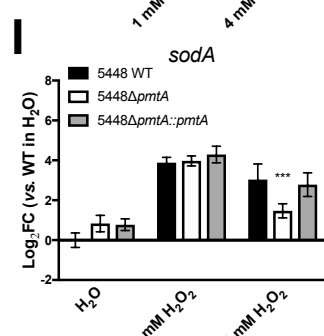
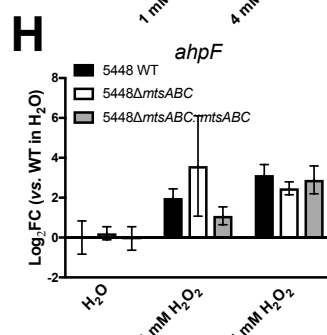
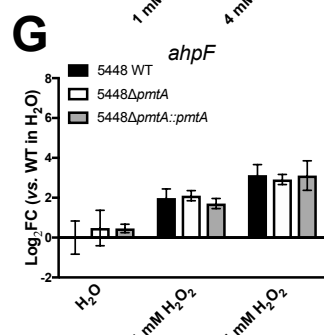
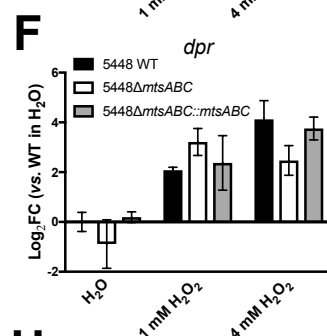
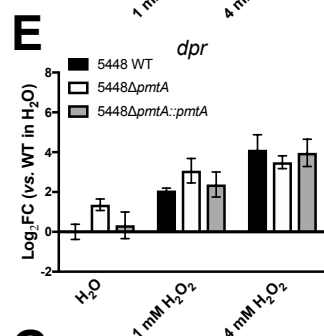
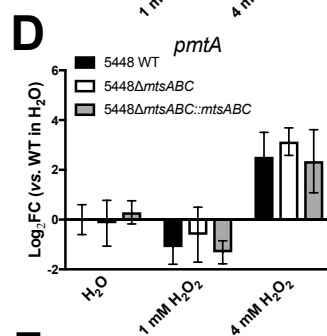
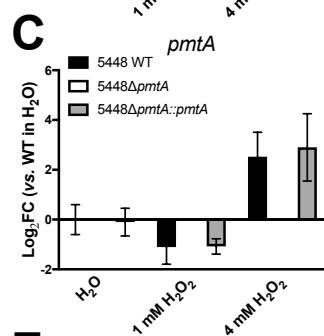
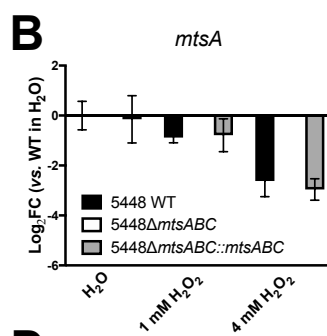
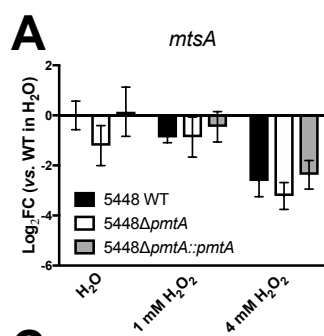
replicates (2-way ANOVA used comparing all to control of that strain \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ ).





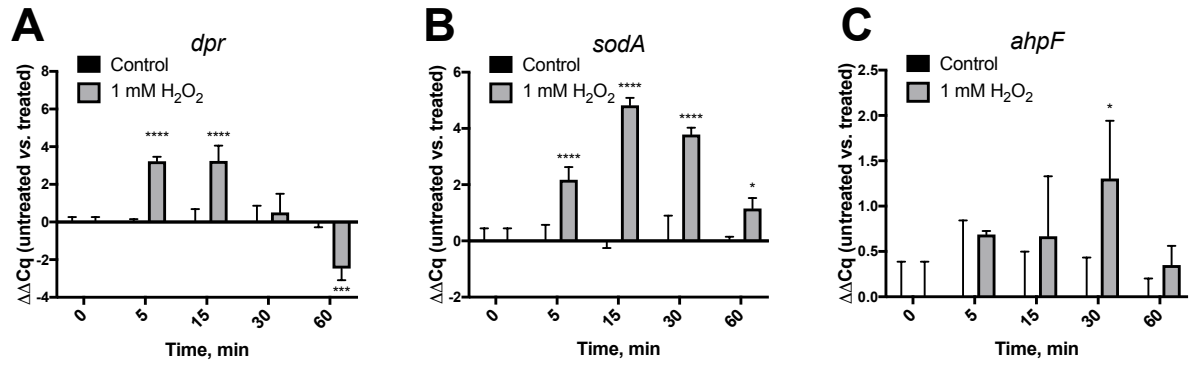
**Figure S4 - Gene expression analysis of PerR-regulated genes in 5448 WT, 5448Δ*perR* and 5448Δ*perR*::*perR* at various phases of growth**

5448 WT, 5448Δ*perR*, and 5448Δ*perR*::*perR* were grown in THY at 37°C and harvested at OD<sub>600</sub> = 0.3 (early), OD<sub>600</sub> = 0.6 (mid), or OD<sub>600</sub> = 0.8 (late) -exponential phase. Gene expression of *pmtA* (A), *ahpC* (B) and *dpr* (C) was analysed by qPCR using *gyrA* as the reference gene. Data represents the mean ± standard deviation of 3 independent biological replicates (2-way ANOVA used comparing all to control of that strain \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ ). Figure S4D in main manuscript.



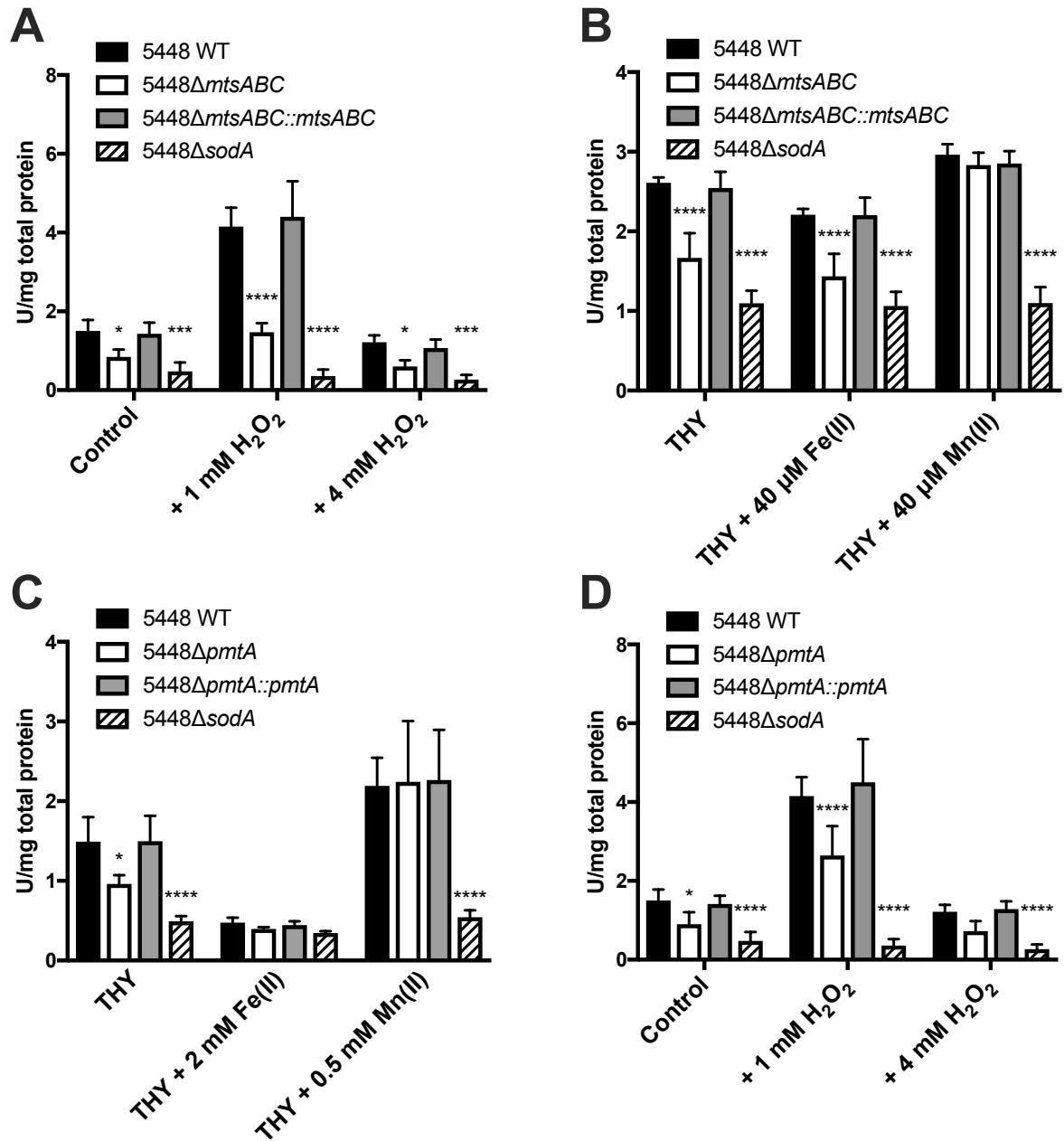
### Figure S5 - Gene expression analysis during hydrogen peroxide stress

5448 WT, 5448 $\Delta$ *mtsABC*, 5448 $\Delta$ *mtsABC::mtsABC*, 5448 $\Delta$ *pmtA* and 5448 $\Delta$ *pmtA::pmtA* were grown at 37°C to mid-exponential phase ( $OD_{600}$  = 0.6-0.8) in THY and challenged with either sterile H<sub>2</sub>O, 1 mM H<sub>2</sub>O<sub>2</sub> or 4 mM H<sub>2</sub>O<sub>2</sub> and gene expression analysed by qPCR using *gyrA* as the reference gene. Data represents the mean  $\pm$  standard deviation of 3 independent biological replicates. 2-way ANOVA used comparing strains to 5448 WT of that treatment (\*  $P < 0.05$ , \*\*  $P < 0.001$ ). Figure S5K in main manuscript.



**Figure S6 – Gene expression timecourse analysis during hydrogen peroxide stress**

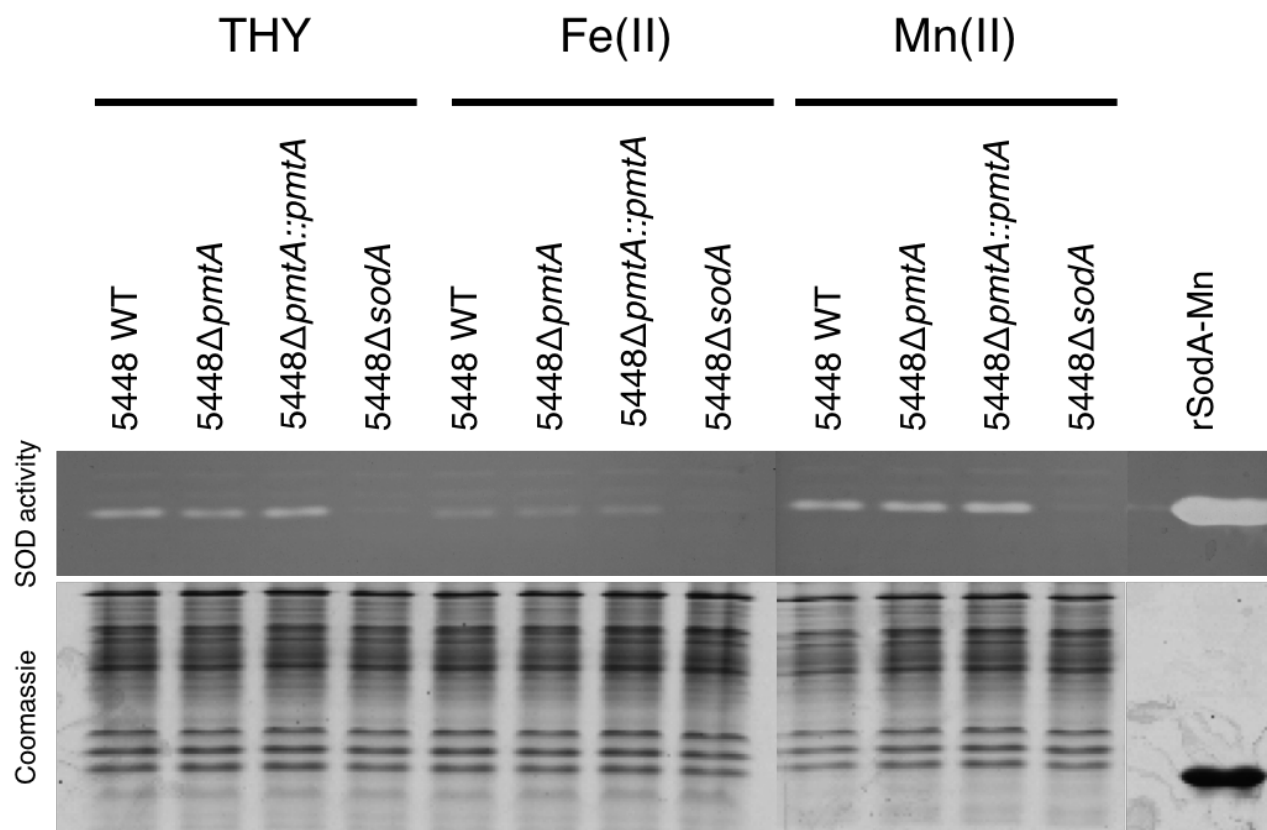
5448 WT was grown at 37°C to mid-exponential phase ( $OD_{600} = 0.6-0.8$ ) in THY and challenged with either sterile H<sub>2</sub>O (control) (black bars) or 1 mM H<sub>2</sub>O<sub>2</sub> (grey bars) and gene expression analysed at t = 0, 5, 15, 30 or 60 min by qPCR using *gyrA* as the reference gene. Data represents the mean  $\pm$  standard deviation of 3 independent biological replicates. Figure S6D in main manuscript.



**Figure S7 - Superoxide dismutase activity**

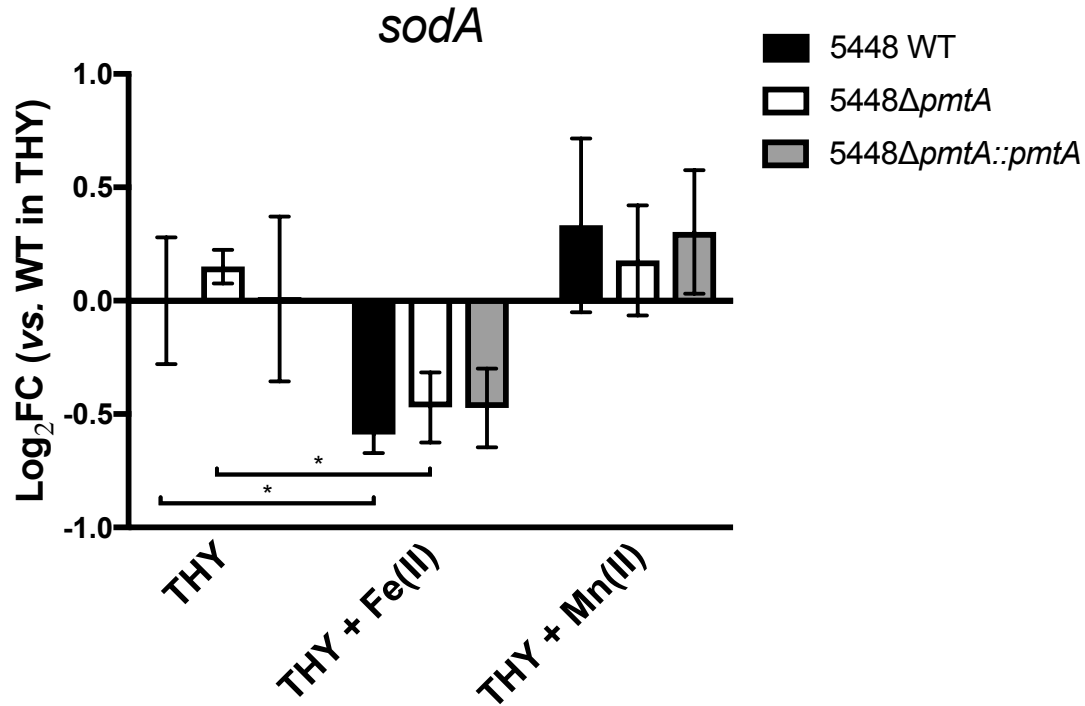
5448 WT, 5448Δ*mtsABC*, 5448Δ*mtsABC::mtsABC* and 5448Δ*sodA* (A) or 5448 WT, 5448Δ*pmtA*, 5448Δ*pmtA::pmtA* and 5448Δ*sodA* (D) were grown in THY at 37°C to mid-exponential phase (OD<sub>600</sub> 0.6 – 0.8), challenged with either H<sub>2</sub>O, 1 mM H<sub>2</sub>O<sub>2</sub> or 4 mM H<sub>2</sub>O<sub>2</sub> cells harvested and SOD activity analysed. 5448 WT, 5448Δ*mtsABC*, 5448Δ*mtsABC::mtsABC* and 5448Δ*sodA* were grown in THY, THY + 40 μM Fe(II), or THY + 40 μM Mn(II) to mid-exponential growth phase (OD<sub>600</sub> 0.6 – 0.8), cells harvested and SOD activity analysed (B).

5448 WT, 5448 $\Delta$ *pmtA*, 5448 $\Delta$ *pmtA::pmtA* and 5448 $\Delta$ *sodA* were grown in THY, THY + 2 mM Fe(II) or THY + 0.5 mM Mn(II) to mid-exponential growth phase (OD<sub>600</sub> 0.6 – 0.8), cells harvested and SOD activity analysed (C) (2-way ANOVA of strains compared to 5448 WT of that condition, \*  $P < 0.05$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ ).



**Figure S8 – SOD in-gel activity assay and coomassie gel**

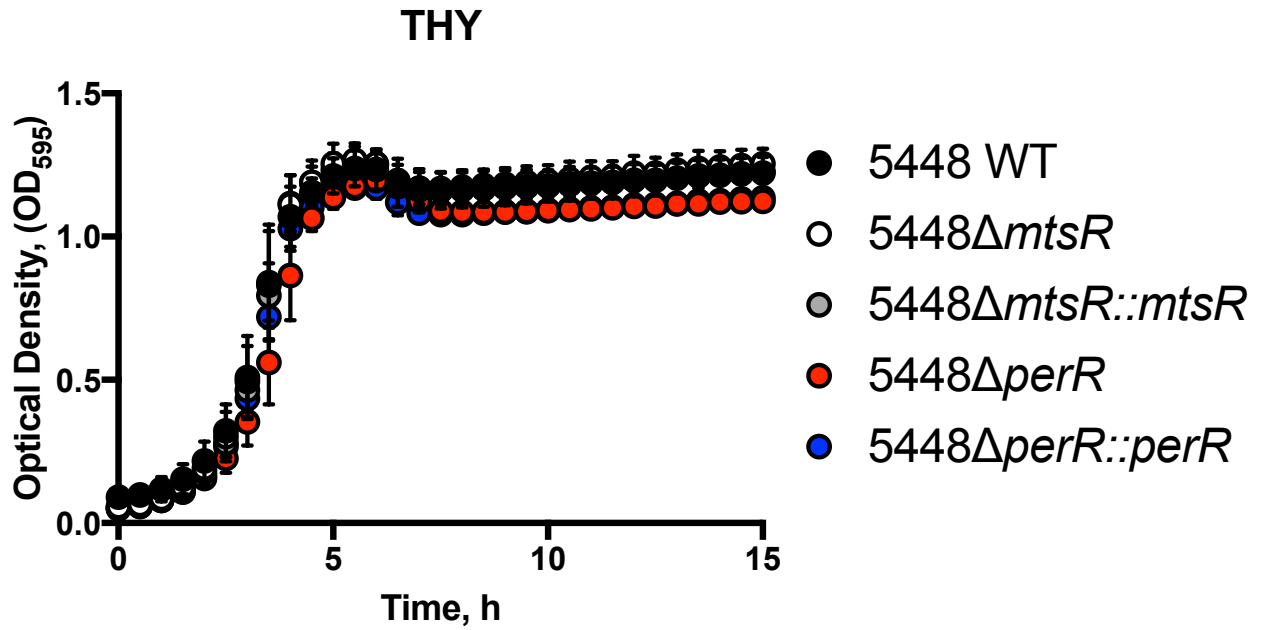
5448 WT, 5448Δ*pmtA* and 5448Δ*pmtA::pmtA* were grown in THY, THY + 2 mM Fe(II) or THY + 0.5 mM Mn(II) to mid-exponential growth phase (OD<sub>600</sub> 0.6 – 0.8), cells harvested and mechanically lysed. 40 µg protein was loaded into native gels and in-gel SOD activity analysed (top) or stained with coomassie for total protein (bottom).



**Figure S9 – Gene expression analysis of *sodA***

Gene expression analysis of *sodA*. 5448 WT, 5448Δ*pmtA* and 5448Δ*pmtA*::*pmtA* were grown at 37°C to mid-exponential phase ( $OD_{600} = 0.6-0.8$ ) in either THY alone, THY + 2 mM Fe(II) or 0.5 mM Mn(II) and gene expression of *sodA* analysed by qPCR using *gyrA* as the reference gene. Data represents the mean  $\pm$  standard deviation of 3 independent biological replicates.





**Figure S10 - Growth of strains in THY for streptonigrin control**

5448 WT (black circles), 5448Δ*mtsR* (white circles), 5448Δ*mtsR*::*mtsR* (grey circles), 5448Δ*perR* (red circles) and 5448Δ*perR*::*perR* (blue circles) were diluted to OD<sub>600</sub> = 0.05 into THY broth. Growth was monitored at 37°C by optical density recording at 595 nm (OD<sub>595</sub>). Graphs represent mean ± standard deviation of 3 independent biological replicates.

## References

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